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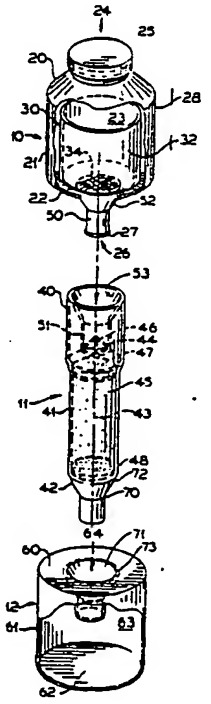
(57) Abstract

An apparatus for detecting a target molecule in a liquid sample suspected of containing the same is disclosed. The invention comprises a sample module (10) and a reaction module (11). The sample module (10) has a liquid containing sample chamber (23), a sample chamber inlet opening (24), and a sample chamber outlet opening (26) formed therein. The reaction module (11) has a reaction chamber (43), a reaction chamber inlet opening (44), and a reaction chamber outlet opening (64) formed therein. A ligand which binds the target molecule is immobilized in the reaction chamber (43). A frangible barrier (27) is sealably connected to the sample chamber outlet opening (26) and a pointed member (46) is connected to the reaction chamber inlet (44). The sample module (10) and the reaction module (11) are releasably engaged to one another with the sample chamber outlet opening (26) and the reaction chamber inlet opening (44) in fluid communication.



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METHOD AND APPARATUS FOR DETECTING ENVIRONMENTAL CONTAMINANTS

Field of the Invention

The present invention relates to environmental testing in general, and more particularly relates to methods of environmental testing and modular apparatus for carrying out such tests.

Background of the Invention

Each day toxic chemicals are introduced into the environment through the use of insecticides and herbicides in agriculture, the use of solvents and other chemicals by industry, and from leaking underground storage tanks found at automobile service stations and other facilities. Congress and numerous state governments have responded with legislation requiring the cleanup of contaminated sites. The cost of conducting this cleanup, however, is likely to strain the ability of society to pay for the environmental quality it desires.

The major expense of cleaning up any contaminated site is the cost of treating or disposing of the contaminated material. These costs have increased dramatically in recent years, and similar increases are predicted for the future.

For contaminated soil, the decontamination of which costs two hundred dollars or more per ton, it is prudent to minimize the quantity of clean, uncontaminated soil removed from a site for disposal or treatment. Yet, at the present time there is no fast, easy way to rapidly differentiate contaminated from uncontaminated soil outside the lab and in the field, and no way of providing rapid on-site mapping of contaminated soil in the field.

U.S. Patent No. 4,425,438 to Bauman et al. discloses an assay device comprised of a clear test tube. A capillary tube is suspended in the test tube by means of a funnel, and a cup is fitted to the top of the tube. Glass beads in the capillary tube carry an analytical absorbent such as biotin or avidin. The funnel contains glass beads which carry a primary absorbent. A test substance and an analytical reagent are placed in the cup and permitted to drain through the funnel and into the capillary tube. The order the test substance and the analytical absorbent are placed in the cup is not critical (Column 12, line 67 et seq.). The test substance may be an antigen, the primary absorbent may be an antibody, and the analytical reagent may be a three-member conjugate of the test substance, a detectable group, and a group which binds to the analytical absorbent. The presence of a test substance in a sample displaces the analytical reagent from the primary absorbent into the analytical absorbent, with the amount of analytical reagent present in the analytical absorbent being proportional to the amount of test substance bound to the primary absorbent. Only a limited quantity of test substance can be processed through the device. Moreover, the passing of excess test substance through the device can lead to interference and variability in the test from the sample matrix.

U.S. Patent No. 4,787,971 to Donald concerns a column chromatography separation device for separating chemicals from an eluent fluid. The apparatus includes a tubular column, a receptacle container, a cap member, and an adapter. The tubular column contains an affinity chromatography media. The

device is adapted for laboratory use, and not field testing for environmental contaminants.

U.S. Patent No. 4,092,408 to Litt discloses a heterogenous assay in which antibody is bound to a support, a biological fluid containing both an antigen and labeled antigen contacted to the support, and unbound labeled antigen then detected. U.S. Patent No. 4,590,157 to Chandler et al. discloses an enzyme-linked immunosorbent assay using urease as the enzyme, urea as the indicator, and dibromo-O-cresolsulfonphthalein as the indicator. Neither of these techniques are adapted to field use.

In view of the foregoing, an object of the present invention is to provide a rapid and convenient method for detecting environmental contaminants in a liquid sample, and apparatus for performing this method, which can be carried out in the field with minimum interference and optimum performance, without the necessity of returning the sample to a laboratory.

A second object of the present invention is to provide a test device in which the sensitivity of the device can be increased by simply increasing the quantity of sample liquid which is passed through the device.

A third object of the present invention is to provide a test device in which the liquid sample matrix does not interfere with the detection step, thereby enabling greater sensitivity and decreased variability in the test.

Summary of the Invention

The foregoing and other objects are achieved by the invention disclosed herein. A first aspect of the present invention is an apparatus for detecting a target molecule in a liquid sample suspected of containing the same. The apparatus comprises a sample module and a reaction module. The sample module has a sample chamber, a sample chamber inlet opening, and a sample chamber outlet opening formed therein. Closure means are operatively associated with the sample chamber outlet opening for retaining a liquid sample in the sample chamber. The reaction module has a reaction chamber

and a reaction chamber inlet opening formed therein. Target molecule detection means are contained within the reaction chamber. Means are provided on the sample module and on the reaction module for joining the modules with the sample chamber outlet opening and the reaction chamber inlet opening in fluid communication. Means are operatively associated with the reaction chamber inlet opening for releasing a liquid sample in the sample chamber past the closure means and into the reaction chamber via the sample chamber outlet opening when the modules are joined.

A second aspect of the present invention is a method of detecting a hapten in a liquid sample suspected of containing the same. The method comprises the following steps: (a) providing an immobilized antibody which binds to the hapten; (b) contacting the liquid sample to the immobilized antibody; (c) removing the liquid sample from contact with the immobilized antibody; then (d) contacting a liquid developer solution to the immobilized antibody, the liquid developer solution containing a labeled molecule which binds to the antibody, with the labeled molecule provided in a quantity such that all of the labeled molecule will bind to the antibody if no hapten was present in the sample; and then (e) detecting the presence or absence of free (i.e., unbound) labeled molecule in the developer solution, with the presence of the unbound labeled molecule of the developer solution indicating the presence of the hapten in the liquid sample.

A third aspect of the present invention is a preferred method of detecting a hapten in a liquid sample suspected of containing the same. The method comprises the following steps: (a) providing a reaction module having a reaction chamber and an antibody which binds to the hapten immobilized in the reaction chamber; (b) passing the liquid sample through the reaction module; followed by (c) bringing an indicator support into fluid-flow association with the reaction module; (d) passing a liquid developer solution through the reaction module and onto the indicator support, the developer solution containing a labeled molecule which binds to the antibody,

with the labeled molecule provided in a quantity such that all of the labeled molecule will bind to the antibody if no hapten was present in the sample; and then (e) detecting the presence or absence of labeled molecule on the indicator support, the presence of the labeled molecule on the indicator support indicating the presence of the hapten in the liquid sample.

Brief Description of the Drawings

FIGURE 1 is an exploded perspective view of an apparatus of the present invention, including a sample module, a reaction module, and a collection module;

FIGURE 2 is an enlarged side sectional view of a sample module and reaction module of the present invention, showing the sample module outlet opening and reaction module inlet opening before being joined;

FIGURE 3 is a top sectional view of a reaction module of the present invention, taken along line 3-3 of Figure 2;

FIGURE 4 is an enlarged side sectional view of a sample module and reaction module similar to Figure 2, except showing the sample module outlet opening and reaction module inlet opening after being joined; and

FIGURE 5 is a perspective view of three (3) reaction modules of the present invention suspended over a test strip, with developer solution being passed through the reaction modules.

Detailed Description of the Preferred Embodiment

As shown in Figure 1, a first embodiment of the present invention comprises a sample module 10, a reaction module 11, and a collection module 12. The modules are constructed of any suitable hard, substantially impermeable, non-reactive material, such as polycarbonate. The apparatus is useful for detecting the presence of any target molecule in a liquid sample, but is preferably employed for detecting haptens in a liquid sample. Many common environmental contaminants are aromatic or aliphatic hydrocarbons which are haptens. Exemplary of such haptens are benzene, toluene, xylene, aldicarb, benzo (a) pyrene, and pentachlorophenol.

The sample module 10 comprises a hollow cylinder member having a top portion 20, a body portion 21, and a bottom portion 22. The body portion has a collection chamber 23 formed therein, the top portion has a collection chamber inlet opening 24 formed therein (covered by a cover 25 as illustrated), and the bottom portion has a collection chamber outlet opening 26 formed therein. The inlet opening has a threaded lip to which a threaded cover may be joined. A frangible, fluid impermeable barrier 27 sealably connected to the collection chamber outlet opening 26 serves as a closure means operatively associated with the sample chamber outlet opening for retaining a liquid sample in the sample chamber. The frangible barrier illustrated comprises an impermeable element (such as a metallic foil) secured to the bottom portion of the sample module with a suitable water impermeable adhesive so as to completely overlie the outlet opening.

The sample module has an internal cylinder 30 which serves as means formed in the collection chamber for delivering a measured quantity of liquid through the outlet opening. The circumference of the internal cylinder is less than the circumference of the collection module, so that a space 31 is defined between the internal cylinder side wall 32 and the collection chamber body portion 21. The height of the internal cylinder side wall 32 is less than the height of the collection chamber body portion 21. The internal cylinder is joined to the bottom portion 22 of the sample module, but not to the top portion 20 of the sample module, so that liquid may flow through the inlet opening 24 and into the internal cylinder 30. The volume of the internal cylinder 30 is calibrated to deliver a predetermined amount of the liquid sample being tested through the outlet opening 26 when the internal cylinder is completely filled. Excess liquid placed in the sample module flows over the top 33 of the internal cylinder side wall into the space 31. Alternative means for delivering a predetermined volume of liquid sample from the sample module are to fill the entire volume of the sample module 10 with the liquid sample, (with the chamber volume

being sized as appropriate) or to fill the sample chamber 23 with the liquid sample up to a preset indicia on the side wall 28 of the sample chamber. A porous filter 34 positioned in the internal cylinder serves as filter means for filtering a liquid sample contained in the sample module prior to passing the sample out of the module through the outlet opening.

The reaction module 11 comprises a tubular column member having a top portion 40, a body portion 41, and a bottom portion 42. The body portion 41 has a reaction chamber 43 formed therein, the top portion 40 has a reaction chamber inlet opening 44 formed therein, and the bottom portion has a reaction chamber outlet opening 45 formed therein. The volume of the reaction chamber 43 is preferably not more than the volume of the predetermined liquid sample delivered by the sample module. A pointed member 46 connected to the reaction chamber, disposed within the inlet opening 44 (see Figure 3) and positioned to rupture the sample chamber frangible barrier 27, serves as a means operatively associated with the reaction chamber inlet opening 44 for releasing the liquid sample past the closure means and into the reaction chamber via the sample chamber outlet opening when the sample module and reaction module are joined (see Figures 2 and 4).

Numerous other closure means and means for releasing the liquid sample are available which would perform equally well in the present invention. For example, a one-way valve or a pop-out insert could be used as the closure means, and a blunt probe could be used as the means for releasing the liquid sample. Still other alternate embodiments will be appreciated by those skilled in the art.

A ligand which binds the target molecule is immobilized in the reaction chamber 43, and serves as a target molecule detection means contained within the reaction chamber. The term "ligand," as used herein, means any molecule which binds selectively to a target molecule with the two together forming a specific binding pair. The term "immobilized," as used herein, means merely that the ligand stays within the reaction module. The ligand is preferably immobilized in the reaction

chamber by binding it to a solid support 45, with the solid support in turn itself immobilized in the reaction chamber. Any solid support can be used, exemplary solid supports including agarose, starch, cellulose, chitin, collagen, synthetic polymers of latex or acrylic derivatives, and inorganic materials such as ceramic and porous silica. Optimally, the support is not reactive with the sample compound and should not non-specifically bind. Suitable ligands include, but are not limited to, antibodies (both monoclonal and polyclonal), peptides, polynucleic acids, antitoxins, chelating agents, enzyme inhibitors, receptor agonists, receptor antagonists, transport proteins, avidin and biotin. Antibodies and chelating agents are preferred. Suitable chelating agents include ethylenediaminetetraacetic acid (EDTA), phosphonoacetic acid, pyrophosphate, dibasic orthophosphate, and crown ethers such as dicyclohexano-18-crown-6, cyclodexetrins, and cryptands. The chelating agents are preferably used to detect metal ions in solutions suspected of containing the same. The ligand is bound to the solid support by any suitable binding, including both chemical or physical binding, but is preferably bound to the solid support covalently.

For detecting haptens, the ligand is preferably an antibody. The method of the present invention is advantageously employed with antibodies having affinity constants of about 10^8 liters per mole or less, and is particularly advantageously employed with antibodies having affinity constants of about 10^7 liters per mole or less.

In the illustrated embodiment, the sample chamber contains a particulate solid support 45 (e.g., silica) to which the ligand is bound. Filter screens 46, 47 joined to the interior side wall of the reaction chamber body portion are included at the upper and lower ends of the reaction chamber to secure the solid support within the reaction chamber, and provide means for immobilizing the solid support in the reaction chamber.

Those skilled in the immunoassay art will appreciate numerous other equally suitable ways to detect the target molecule which comprise alternate embodiments of the present invention. For example, the target molecule could combine with other reagents or ligands in the reaction chamber and pass out through the reaction chamber outlet opening for ultimate collection and/or completion of the detection step.

As shown in detail in Figures 2 through 4, the bottom portion of the sample module has a downwardly projecting nipple 50 and the top portion of the reaction module has an upwardly facing bore 51 configured to receive the downwardly projecting nipple. The nipple 50 and bore 51 serve as means formed on the sample module and on the reaction module for joining the modules with the sample chamber outlet opening 26 and the reaction chamber inlet opening 44 in fluid communication. The nipple 50 and bore 51 have tapered portions 52, 53 which mate with one another to assist in connecting the two modules to one another. Preferably, the outer diameter of the nipple and the inner diameter of the bore are sized so that, when joined, the nipple and bore frictionally engage one another. Other shapes and arrangements of elements for joining the modules are also suitable, so long as they provide for fluid communication from the sample chamber to the reaction chamber via the sample chamber outlet opening.

The collection module 12 has a top portion 60, 63, body portion 61, and closed bottom portion 62. The body portion 61 has a collection chamber 63 formed therein and the top portion has a collection chamber inlet opening formed therein. Preferably, the collection chamber volume is at least as great as the reaction chamber volume, and is more preferably at least twice as great as the reaction chamber volume. The bottom portion of the reaction module has a downwardly projecting nipple 70 and the top portion of the collection module has an upwardly facing bore 71 configured to receive the downwardly projecting nipple. The nipple 70 and bore 71 serve as means formed on the reaction module and on the

collection module for joining these modules with the reaction chamber outlet opening and the collection chamber inlet opening in fluid communication. The nipple and bore have tapered portions 72, 73 which mate with one another to assist in connecting the two modules to one another. As previously, the outer diameter of the nipple and the inner diameter of the bore are preferably sized so that, when joined, the nipple and bore frictionally engage one another.

If desired, the reaction module can be provided filled with a liquid to hydrate the solid support. In this case, a removable cap would be connected to the top portion 40 of the reaction module 11. A frangible, fluid impermeable barrier would then be sealably connected to the reaction chamber outlet opening 45, analogous to the barrier 27 sealably connected to the collection chamber outlet opening 26. The collection module 12 could then be provided with a pointed member connected to the collection chamber inlet opening 64 and positioned to rupture the reaction chamber frangible barrier, all analogous to the pointed member 46 of the reaction chamber.

The operation of the apparatus of the preferred embodiment is best illustrated by Figures 2 and 4. A liquid sample to be tested for the presence or absence of a target molecule is first placed in the sample module 10. The sample chamber outlet opening 26 is then joined to the reaction chamber inlet opening 44, whereby the frangible barrier 27 is ruptured by the pointed member 46 and the liquid sample thereby drained from the sample chamber 23 into the reaction chamber 43. The presence or absence of target molecule in the reaction chamber is then determined.

The detection method of the present invention is exemplified by Figure 5. In the illustrated embodiment, a liquid sample suspected of containing the hapten is first passed through a reaction module. The reaction chamber of the reaction module 11 contains an antibody which binds the hapten. The reaction module 11 is then suspended on a rack 75 over an indicator strip 76 and a liquid developer solution

77 passed through the reaction module onto the test strip. The developer solution contains a labeled molecule which binds to the antibody immobilized in the reaction chamber. The developer solution is provided in a quantity such that all of the labeled molecule will bind to the antibody if no hapten was present in the sample. Thus, if no hapten was present in the liquid sample, then the labeled molecule will not pass through the reaction chamber and onto the test strip. However, if hapten was in fact present in the liquid sample, then at least some of the labeled molecule will pass through the reaction chamber and onto the test strip.

In an embodiment of the foregoing method, the proportion of antibody to labeled molecule in the liquid developer solution is increased by a predetermined amount so that, if hapten is present in the liquid sample but the quantity of the hapten present is below a predetermined level, then all of the labeled molecule will bind to the antibody, none of the labeled molecule will be free, and no indication of the presence of the hapten in the liquid sample will be given. Thus, for example, if one is only concerned with detecting benzene in a sample at concentrations of five parts per billion or more, then the proportion of antibody to labeled molecule is increased so that, for a given quantity of sample liquid, all of the labeled molecule is bound to the antibody even though some antibody is occupied by the benzene. If the benzene concentration exceeds five parts per billion, then the quantity of labeled molecule is such that the antibody binding capacity is exceeded and some of the labeled molecule cannot bind antibody and will remain free and an indication of the presence of benzene in the liquid sample will be given when, for example, the labeled molecule contacts the indicator strip.

The labeled molecule in the developer solution preferably comprises a hapten and a detectable or signal producing group bound to the hapten. The hapten of the labeled molecule may be the same as the hapten for which detection is sought. The detectable group may be any

conventional detectable group, such as an enzyme label, a radioisotope label, a fluorescent label, or a dye polymer. In the preferred embodiment, the detectable group comprises an enzyme, the readout strip comprises a paper strip with an enzyme substrate of the enzyme impregnating the paper strip, and the enzyme and enzyme substrate are selected so that the reaction product of the enzyme and substrate is one which provides a positive color change on the readout strip (the color of the paper strip itself is likewise selected to achieve this result, with the paper strip preferably being a white paper strip). Thus, a color change on the indicator support indicates that the target molecule was present in the liquid sample, and the absence of a color change on the indicator support indicates that the target molecule was not present in the liquid sample. A preferred labeled hapten is a benzene-beta-galactosidase conjugate, and a preferred enzyme substrate is orthonitrophenyl-beta-D-galactopyranoside (ONPG). Beta-galactosidase converts colorless ONPG into a colored ortho-nitrophenyl product. A variety of other substrates are also available which provide the capability of producing different colored reactions (e.g., X-gal., CPRG) or fluorescent reactions (e.g., methyl-umbeliferone-β-D-galactopyranoside (MUG)). An alternate labeled hapten is a benzene-horseradish peroxidase (HRP) conjugate. In this case, the developer contains a chromophore (e.g., orthophenylene diamine) and the readout strip could be paper impregnated with a peroxidase substrate (e.g., hydrogen peroxide, urea peroxide). In the presence of HRP, the peroxidase substrate donates an electron to the chromophore, which then changes color.

Numerous variations can be made on the foregoing. Alternate indicator supports can be used, including particles, beads, and solid cards. The indicator support can be brought into fluid-flow association with the reaction chamber by any technique which permits movement of the liquid developer solution from the reaction chamber to the indicator support. Preferably, fluid-flow association is established by bringing

the indicator support to a position which permits the gravity drainage of the developer solution from the reaction chamber onto the solid support. Alternatively, however, the developer solution could be moved from the reaction chamber onto an appropriately oriented indicator support by centrifugal force, or the indicator support could be immersed in developer solution in the reaction chamber.

The present invention is particularly advantageous for detecting haptens in soil and water samples. When a liquid leachate sample is taken, it will be contaminated by particulate organic and inorganic matter, and the pH may vary from sample to sample. The present invention, by separating the liquid sample matrix from the ligand in the manner described prior to introducing the developer, reduces interference of the antibody and developer conjugate in the binding assay.

Binding events have, for clarity, been described in absolute terms herein (i.e., "all bound," "none bound"). In practice, it will be appreciated that minor deviations from the absolute will occur without altering or detracting from the operation of the present invention.

The foregoing is illustrative of the present invention, and is not to be taken as restrictive thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. An apparatus for detecting a target molecule in a liquid sample suspected of containing the same, comprising:

(a) a sample module having a sample chamber, a sample chamber inlet opening, and a sample chamber outlet opening formed therein;

(b) closure means operatively associated with said sample chamber outlet opening for retaining a liquid sample in said sample chamber;

(c) a reaction module having a reaction chamber and a reaction chamber inlet opening formed therein;

(d) target molecule detection means contained within said reaction chamber;

(e) means formed on said sample module and on said reaction module for joining said modules with said sample chamber outlet opening and said reaction chamber inlet opening in fluid communication; and

(f) means operatively associated with said reaction chamber inlet opening for releasing said liquid sample past said closure means and into said reaction chamber via said outlet opening.

2. An apparatus as claimed in Claim 1, further comprising means formed in said sample chamber for delivering a measured quantity of said liquid sample to said reaction chamber.

3. An apparatus as claimed in Claim 1, wherein said closure means comprises a frangible barrier sealably connected to said sample chamber outlet opening, and wherein said means operatively associated with said reaction chamber inlet opening for releasing said liquid sample comprises a pointed member connected to said reaction chamber, said pointed member disposed within said reaction chamber inlet opening and positioned to rupture said frangible barrier when said modules are joined.

4. An apparatus as claimed in Claim 1, further comprising a collection module having a collection chamber and a collection chamber inlet opening formed therein, and means operatively associated with said reaction chamber outlet opening and with said collection chamber inlet opening for releasably engaging said chambers to one another.

5. An apparatus as claimed in Claim 1, wherein the volume of said sample chamber is greater than the volume of said reaction chamber.

6. An apparatus as claimed in Claim 1, wherein said reaction module comprises a tubular column member having a top portion, a body portion, and a bottom portion, with said reaction chamber inlet opening formed in said top portion, said reaction chamber formed in said body portion, and said reaction chamber outlet opening formed in said bottom portion, and with said reaction chamber inlet opening and said reaction chamber outlet opening axially aligned with said elongate tubular column member.

7. An apparatus as claimed in Claim 1, wherein said target molecule detection means comprises a solid support immobilized within said reaction chamber and a ligand which binds said target molecule bound to said solid support.

8. An apparatus as claimed in Claim 7, wherein said ligand comprises an antibody.

9. An apparatus as claimed in Claim 8, wherein said antibody comprises an anti-hapten antibody.

10. A sample module useful in an apparatus for detecting a target molecule in a liquid sample suspected of containing the same, said sample module comprising:

(a) a hollow cylinder member having a top portion, a body portion, and a bottom portion, with said body portion having a collection chamber formed therein, said top portion having a collection chamber inlet opening formed therein, and said bottom portion having a collection chamber outlet opening formed therein; and

(b) closure means operatively associated with said sample chamber outlet opening for retaining a liquid sample in said sample chamber.

11. A sample module as claimed in Claim 10, further comprising means formed in said collection chamber for delivering a measured quantity of liquid through said outlet opening.

12. A sample module as claimed in Claim 10, further comprising filter means contained within said collection chamber for filtering said liquid sample before said liquid sample passes through said outlet opening.

13. A sample module as claimed in Claim 10, wherein said closure means comprises a frangible barrier sealably connected to said sample chamber outlet opening.

14. A reaction module useful in an apparatus for detecting a small molecule in a liquid sample suspected of containing the same, said reaction module adapted for use with a sample module having a sample chamber, a sample chamber inlet opening, and a sample chamber outlet opening formed therein, said sample module having closure means operatively associated with said sample chamber outlet opening for retaining a liquid sample in said sample chamber, said reaction module comprising:

(a) a tubular column member having a top portion, a body portion, and a bottom portion, with said body portion having a reaction chamber formed therein, said top portion having a reaction chamber inlet opening formed therein, and said bottom portion having a reaction chamber outlet opening formed therein;

(b) target molecule detection means contained within said reaction chamber; and

(c) means operatively associated with said reaction chamber inlet opening for releasing said liquid sample past said closure means and into said reaction chamber via said sample chamber outlet opening when said sample module and said reaction module are joined.

15. A reaction module as claimed in Claim 14, wherein said closure means comprises a frangible barrier sealably connected to said sample chamber outlet opening, and wherein said means operatively associated with said reaction chamber inlet opening comprises a pointed member connected to said reaction chamber, said pointed member disposed within said opening and positioned to rupture said frangible barrier.

16. a reaction module as claimed in Claim 14, wherein said target molecule detection means comprises a solid support immobilized within said reaction chamber and a ligand which binds said target molecule bound to said solid support.

17. A reaction module as claimed in Claim 16, wherein said ligand comprises and antibody.

18. A reaction module as claimed in Claim 17, wherein said antibody comprises an anti-hapten antibody.

19. A reaction module as claimed in Claim 18, wherein said anti-hapten antibody binds to a hapten selected from the class consisting of consisting of benzene, toluene, xylene, aldicarb, benzo (a) pyrene, and pentachlorophenol.

20. A method of detecting a hapten in a liquid sample suspected of containing the same, said method comprising the steps of

(a) providing an immobilized antibody which binds to said hapten;

(b) contacting said liquid sample to said immobilized antibody;

(c) removing said liquid sample from contact with said immobilized antibody; then

(d) contacting a liquid developer solution to said immobilized antibody, said liquid developer solution containing a labeled molecule which binds to said antibody, with said labeled molecule provided in a quantity such that all of said labeled molecule will bind to said antibody if no hapten was present in said sample, and then

(d) detecting the presence or absence of unbound labeled molecule in the developer solution, the presence of unbound labeled molecule in the developer solution indicating the presence of said hapten in said liquid sample.

21. A method according to Claim 20, wherein said labeled molecule is provided in a quantity so that, if not more than a predetermined amount of hapten is present in said liquid sample, then all of said labeled molecule will bind to said immobilized antibody and no hapten will be indicated as present in said solution.

22. A method according to Claim 20, wherein said labeled molecule comprises a molecule which selectively binds to said ligand and a detectable group bound thereto, said detectable group selected from the class consisting of enzyme labels, fluorescent labels, and radioisotope labels.

23. A method according to Claim 20, wherein said detectable group comprises an enzyme label, and wherein said indicator support carries an enzyme substrate, said enzyme label and said enzyme substrate selected so that the reaction product thereof provides a positive color change on said indicator support.

24. A method according to Claim 20, wherein said hapten is selected from the class consisting of benzene, toluene, xylene, aldicarb, benzo (a) pyrene, and pentachlorophenol.

25. A method of detecting a target molecule in a liquid sample suspected of containing the same, said method comprising the steps of:

(a) providing a reaction module having a reaction chamber and a ligand which binds to said target molecule immobilized in said reaction chamber;

(b) passing said liquid sample through said reaction module; followed by

(c) bringing an indicator support into fluid-flow association with said reaction module;

(d) passing a liquid developer solution through said reaction module and onto said indicator support, said developer solution containing a labeled molecule which binds to said ligand, said labeled molecule provided in a quantity such that all of said labeled molecule will bind to said ligand if no target molecule was present in said sample; and then

(e) detecting the presence or absence of labeled molecule on said indicator support, the presence of said labeled molecule on said indicator support indicating the presence of target molecule in said liquid sample.

26. A method according to Claim 25, wherein said labeled molecule is provided in a quantity so that, if not more than a predetermined amount of hapten is present in said liquid sample, then all of said labeled molecule will bind to said immobilized antibody and no hapten will be indicated as present in said solution.

27. A method according to Claim 25, wherein said labeled molecule comprises a molecule which selectively binds to said ligand and a detectable group bound thereto, said detectable group selected from the class consisting of enzyme labels, fluorescent labels, radioisotope labels, and dye polymers.

28. A method according to Claim 25, wherein said detectable group comprises an enzyme label, and wherein said indicator support carries an enzyme substrate, said enzyme label and said enzyme substrate selected so that the reaction product thereof provides a positive color change on said indicator support.

29. A method according to Claim 25, wherein said hapten is selected from the class consisting of benzene, toluene, xylene, aldicarb, benzo(a)pyrene, and pentachlorophenol.

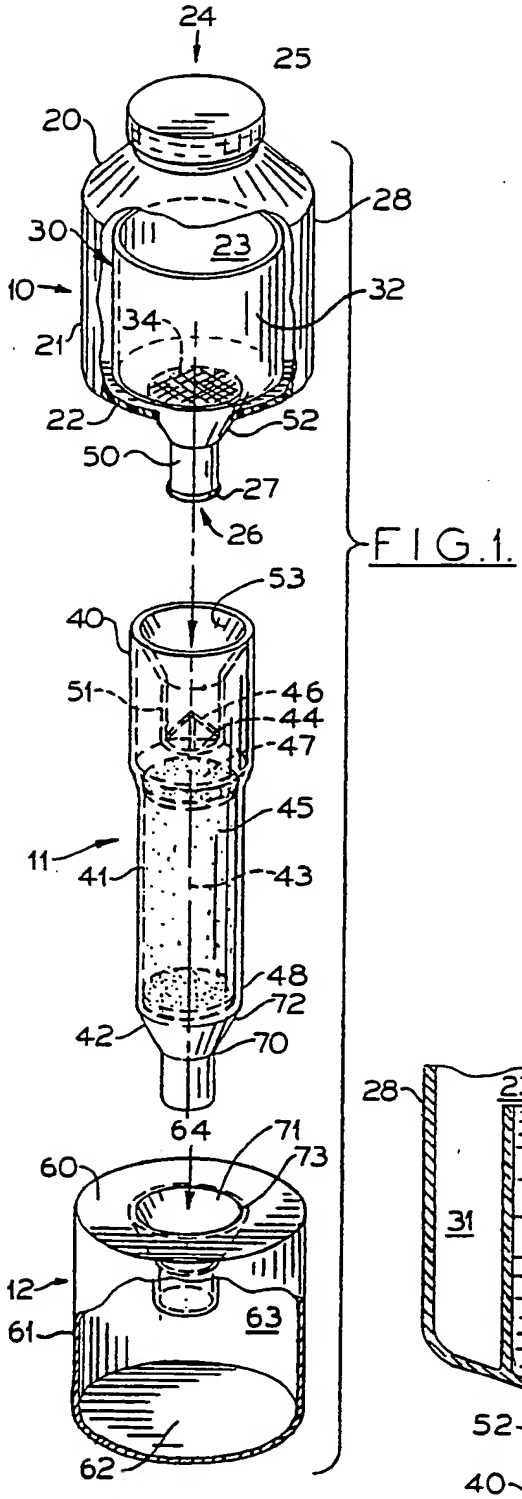

FIG.1.

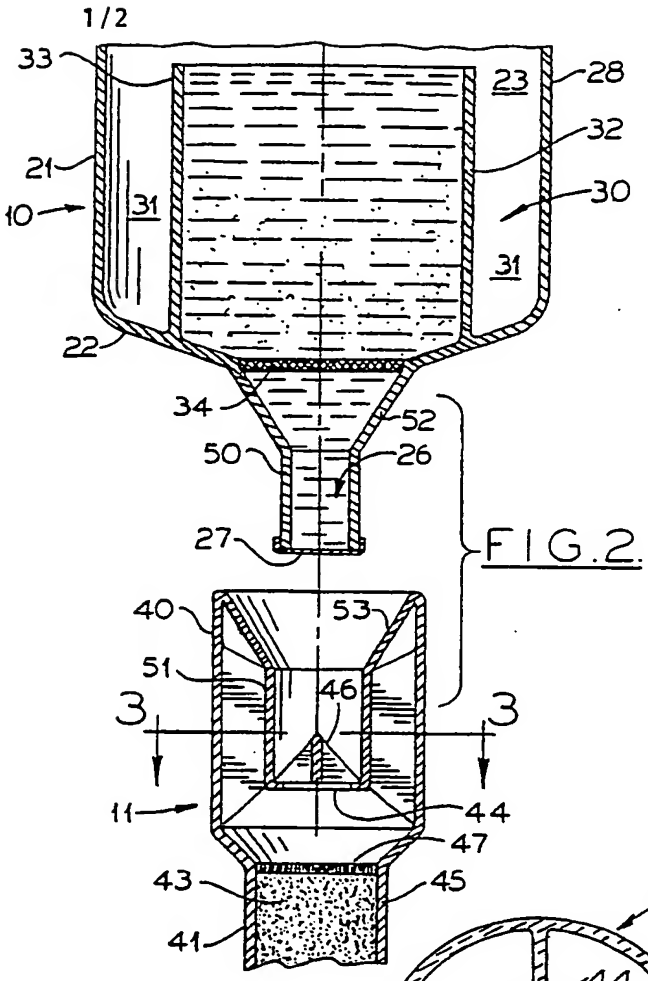

FIG.2.

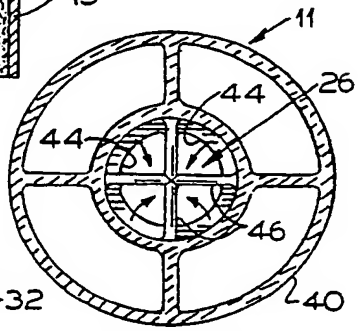

FIG.3.

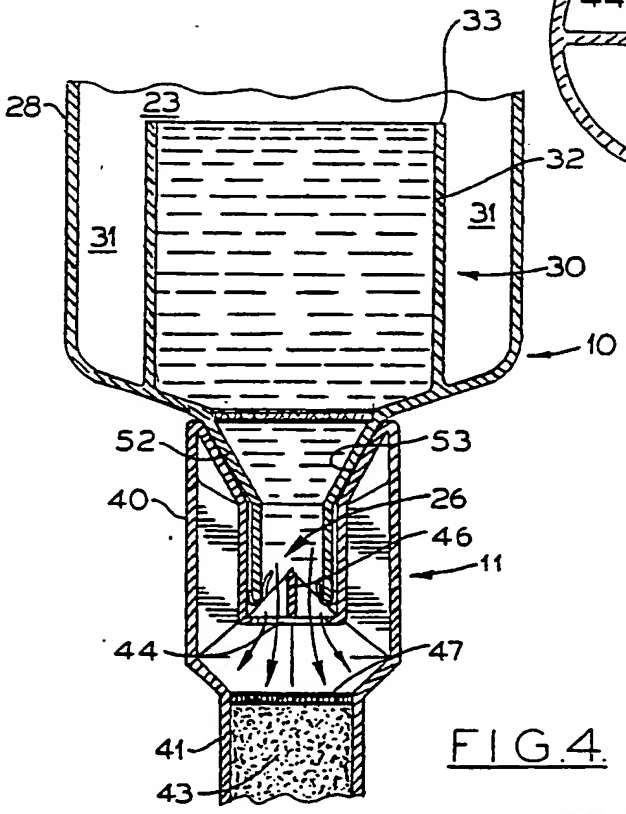

FIG.4.

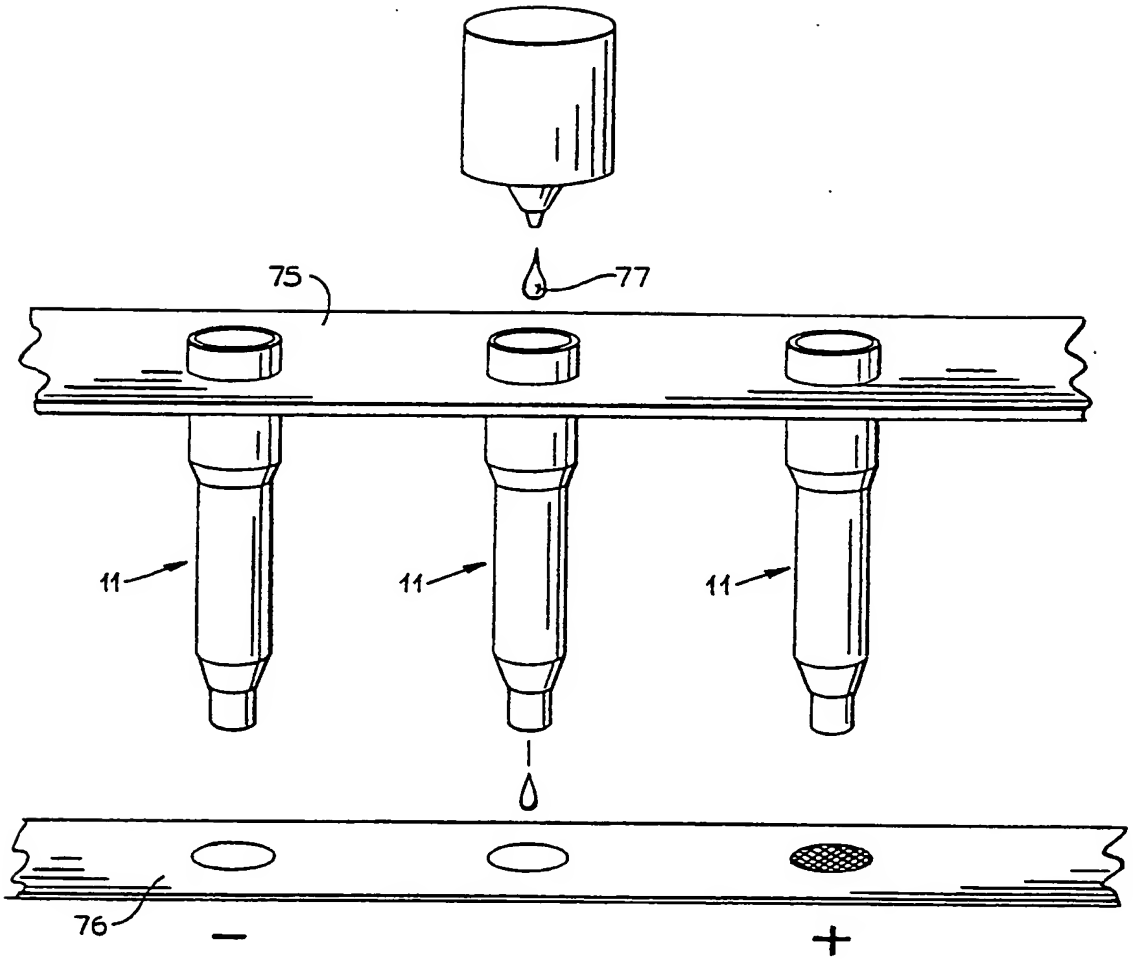

FIG.5.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02817

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): B01L 11/00; C12Q 1/64; G01N 33/53, 33/543, 33/544. 33/545, 33/551
U.S.Cl.: 422/58,59,61,69,101; 435/7,9,299,311; 436/501,518,528,531 33/566

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U S	422/58,59,61,69,101 435/7,9,810,299,311 436/501,518,524,528,531,807,810

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X 1	US, A, 4,476,093 (WATANABE et al.) 09 October 1984 See Figure 1; Abstract; column 2, lines 5-11.	14, 16, 17
X	US, A, 3,640,267 (HURTIG et al.) 08 February 1972 See Figure 1; column 1, lines 61-66, 72-75; column 3, lines 5-16.	10, 11, 13
X Y	US, A, 4,608,231 (WITTY et al) 26 August 1986 See abstract; column 2, lines 23-34. Column 5, line 43 - column 6, line 7.	20, 22 21, 23, 25-28
X Y	US, A, 4,208,187 (GIVNER) 17 Jne 1980 See Figures 7, 7A; column 11, lines 23-65; column 12 lines 10-13; column 12, line 59 - column 13, line 47.	10, 11, 13 1-3, 5, 7, 8, 12, 14-17, 20, 22-23, 25, 27-28
Y	US, A, 4,665,034 (CHANDLER) 12 May 1987 See Figure 3; column 1, lines 21-22, 48-52; column 2, lines 51-66; claims 1, 2, 4, 5, 9, 11.	1-27
Y	US, A, 3,888,629 (BAGSHAWE) 10 June 1975 See Figure 1; column 2, line 13 - column 3, line 55; column 4, lines 11-38.	1-27

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
21 August 1990	26 SEP 1990
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Carol A. Spiegel

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US, A, 4,632,901 (VALKIRS et al.) 30 December 1986 See column 2; lines 32-60.	20, 22, 23
Y	US, A, 4,775,629 (KUHL et al.) 04 October 1988 See Figures 1-3; column 2, lines 29-63; column 3, line 1 - column 4, line 28.	1, 6, 14, 15
Y	US, A, 4,787,971 (DONALD) 29 November 1988 See Figure 7; column 3, lines 34-43; column 4, lines 52-60; column 5, lines 8-26; column 6, lines 3-24.	1, 6-9, 14-19
A	US, A, 4,324,758 (EISENTRAUT et al.) 13 April 1982 see entire document.	1-29

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . , because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-19 and 25-29 drawn to a first specific binding method of detecting a target molecule and an apparatus designed for said method classified in CLass 435 subclass 7.

II. Claims 20-24 drawn to a second specific binding method for detecting a hapten classified in Class 436 subclass 518.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. TELEPHONE PRACTICE

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	US, A, 4,734,262 (BAGSHAWE) 29 March 1988 See Figure 1-4; column 1, lines 7-11, 55-65; column 2, lines 24-28; column 2, line 54 - column 3, line 49.	1-9, 14-19, 25-29
Y	US, A, 4,778,751 (EL SHAMI et al.) 18 October 1988 See abstract; column 6, lines 20-64; column 21, line 1 - column 22, line 5.	7-9, 16-29

CONTINUATION SHEET

ATTACHMENT TO PCT/ISA/TELEPHONE PRACTICE

The above inventions lack unity under PCT Rule 13 since the second method of group II requires an immobilized antibody (see claim 20) whereas, the first method and apparatus of group II do not (see claims 1 and 25); e.g. a liquid phase comprising excess labeled molecules and labeled molecules complexed with target molecule might be separated by filtration and the amount of complexed label on, but not bound to, filter be detected. Alternately, the second method of group II does not require the sample module, closure means, fluid communication means or shunting means of the apparatus of group I.